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ANTIDIABETIC EFFECT OF A COMPOUND ETHANOLIC EXTRACT OF *ANNONA SQUAMOSA*, *ECLIPTA ALBA*, & *BUTEA MONOSPERMA* IN EXPERIMENTAL ANIMALS

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ABSTRACT: Type 2 diabetes is a chronic metabolic disease that has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. Exercise, diet, and weight control continue to be essential and effective means of improving glucose homeostasis. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine. The present study concise on the effect of a compound ethanolic extract of all three plant parts as leaf extract of *Annona squamosa*, leaf extract of *Eclipta alba* and leaf extract of *Butea monosperma*, on fasting blood sugar levels and serum biochemical analysis in alloxan-induced diabetic rats were investigated. All the concentration of compound extracts produced a significant antidiabetic activity at dose levels 1/5 of their lethal dosest.

INTRODUCTION: Diabetes is a syndrome characterized by deranged carbohydrate metabolism resulting in abnormally high blood sugar level (hyperglycemia). It is caused by hereditary, increasing age, poor diet, imperfect digestion, obesity, sedentary lifestyle, stress, drug-induced, infection in pancreas, hypertension, high serum lipid and lipoproteins, less glucose utilization and other factors. It is estimated that the diabetic patients in India will increase by 195% in the near future [1]. The treatment of diabetes with synthetic drugs is costly and chances of side effects are high.

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For example, long-term use of Exenetide (Byetta) [2] has lead to side effects such as nausea, vomiting, diarrhea, dizziness, headache, jittery feeling and acidity. Sulfonylureas cause abdominal upset, headache and hypersensitivity, while Metformin [3] causes diarrhea, nausea, gas, weakness, indigestion, abdominal discomfort and headache. Thiazolidine-diones has side effects like, upper respiratory infections and sinusitis, headache, mild anemia, retention of fluid in the body which may lead to heart failure and muscle pain.

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.

As the number of people with diabetes multiplies worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two to three-folds than the present rates. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations.

Diabetes is a chronic disease affecting around 2-3% of the population worldwide. Unfortunately, after the introduction of sulfonylurea and metformin about 50 years back no major lead has been obtained in this direction of finding a proper drug for diabetes. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug. Plant extract or different folk plant preparations are being prescribed by the traditional practioners and also accepted by the users for diabetes like for any other diseases in many countries especially in third world countries.

Now-a-days more than 400 plants are being used in different forms for hypoglycaemic effects all the claims practitioners or users are neither baseless nor absolutely. Therefore, a proper scientific evaluation a screening of plant by pharmacological tests followed by chemical investigations is necessary. Some plants having hypoglycemic activity as studied by Nahar [4] like *Trigonella foenum-gracecum* (seed), *Nephoelepsis tuberose* (bulb), *Costus specious* (rhizome), *Plantago ovate* (husk), (bulb), *Hemidesmus indicus* (root), *Allium cepa* (bulb).

MATERIALS AND METHODS: The fresh whole herbs of three plants *Annona squamosa*, *Eclipta alba* and *Butea monosperma* were procured from a local vendor from Bundelkhand region, and were authenticated by comparison with herbarium specimens of the Botany Department, Bundelkhand University of Jhansi, India. A voucher specimen no BU/M.Ph./C.V.-1 is preserved in our research laboratory for future references.

These were washed, dried under shade, sieved through mesh no. 45 and subjected to extraction with 300 ml ethanol (80%) in a Soxhlet apparatus for 72 h. After extraction, the solvent was filtered and then evaporated by Rotavapor. The obtained ethanolic extract was stored at -20 ºC until being used.

Experimental Animals: Male albino rats of (200-250 g) were used throughout the experiments. The animals were procured from Animal House facility, Daksh Institute of Pharmaceutical Science, Chhatarpur (Madhya Pradesh). Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature (22-24 ºC), relative humidity (45-55%) and 12 h dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet (Gold mohur, Lipton India Ltd.) and water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) (Protocol no. DIPS/144/2018).

Sample Collection: Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer (Miles Inc, USA) and glucostix (Bayer diagnostic India Ltd., Baroda).

Experimental Design:

Anti-Diabetic Activity: Fasting blood glucose was determined after depriving food for 16 hrs with free access of drinking water. Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) (Loba Chemie, Bombay; 150 mg/kg; Aruna *et al*., 1999). Alloxan was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >160 mg/dl were included in the study. Treatment with plant extracts was started 72 h after alloxan injection. Blood samples were drawn at different intervals till the end of study (*i.e.* 21st day). Fasting blood glucose estimation and body weight measurement were done on day 0, 7, 14 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from

overnight fasted rats and fasting blood sugar [5] was estimated. Serum was separated and analyzed for serum cholesterol [6], serum triglycerides by enzymatic DHBS colorimetric method [7], serum HDL [8], serum LDL [9], serum creatinine [10], serum urea [11], serum alkaline phosphatase hydrolyzed phenol amino antipyrine method [12] were estimated. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5 µ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination. The photomicrographs of histological studies are presented in **Fig. 1**.

The various groups used in experiment;

- ✓ Group A - Served as normal control and did not receive any treatment.
- ✓ Group B - Served as diabetic control and received Alloxan monohydrate and vehicle (0.2 ml of 2% aqueous gum acacia)
- ✓ Group C - Alloxan monohydrate + Glibenclamide (10 mg/kg, p.o.) and served as Standard.
- ✓ Group D - Alloxan monohydrate + Ethanolic extract (100 mg/kg, p.o.)
- ✓ Group E - Alloxan monohydrate + Ethanolic extract (200 mg/kg, p.o.)
- ✓ Group F - Alloxan monohydrate + Ethanolic extract (400 mg/kg, p.o.)

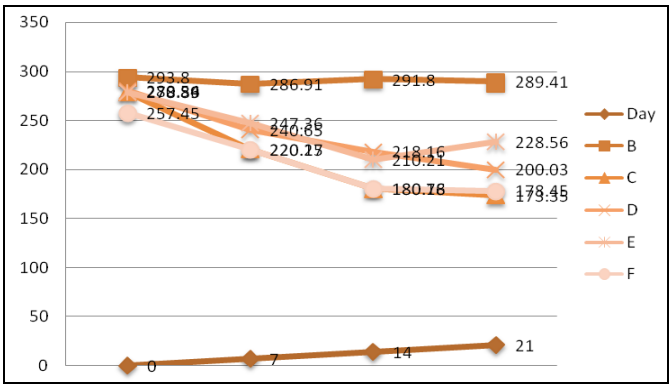

FIG. 1: COMPARATIVE EFFECT OF DIFFERENT PLANTS EXTRACT OF ON BLOOD GLUCOSE LEVEL IN ALLOXAN (150 mg/kg) INDUCED DIABETIS IN RATS

TABLE 1: GROUP TREATMENT FASTING BLOOD GLUCOSE LEVEL (mg/dl) BASAL VALUE 7th DAY, 14th DAY & 21st DAY

Group no.	Treatment	Dose (mg/kg p.o)	Fasting blood glucose level			
			Basal value	7th day	14th day	21st day
A	Vehicle control	0.2 mL(a)	90.46 ± 3.80	92.82 ± 2.92	92.32 ± 1.73	88.29 ± 3.44
B	Diabetic control	0.2 mL(b)	293.8 ± 5.27	286.91 ± 5.05	291.8 ± 5.41	289.41 ± 9.75
C	Alloxan + Glibenclamide control	10 mg/kg	278.86 ± 4.64 *	220.27 ± 6.32*	180.18 ± 5.87*	173.35 ± 4.25
D	Alloxan + Ethanolic extract	100 mg/kg	280.54 ± 3.45	240.65 ± 4.28	218.16 ± 5.98	200.03 ± 6.65
E	Alloxan + Ethanolic extract	200 mg/kg	278.89 ± 3.96	247.36 ± 4.56*	210.21 ± 4.36*	228.56 ± 4.45
F	Alloxan + Ethanolic extract	400 mg/kg	257.45 ± 4.34	220.15 ± 4.35*	180.76 ± 2.52*	178.45 ± 2.61

a Vehicle (0.5% Tween 80 solution in normal saline). b Alloxan single dose of 150 mg/kg i.p in normal saline on day 0. *P<0.05 as compared to vehicle control on corresponding day.

TABLE 2: THE EFFECT OF 3-WEEK TREATMENT WITH VARIOUS EXTRACTS OF THREE PLANTS *ANNONA SQUAMOSA, ECLIPTA ALBA AND BUTEA MONOSPERMA* ON BODY WEIGHT (g) AFTER ALLOXAN (150 mg/kg i.p.) INDUCED DIABETES IN RATS

Group no.	Treatment	Dose (mg/kg p.o)	Average body weight (g) ±SEM			
			Basal value	7th day	14th day	21st day
A	Vehicle control	0.2 mL(a)	202.43 ± 3.24	202.89 ± 1.53	205.24 ± 1.45	207.62 ± 1.98
B	Diabetic control	0.2 mL(b)	203.86 ± 3.85	182.64 ± 7.7∗	171.01 ± 8.5∗	149.67 ± 1.82∗
C	Glibenclamide control	10 mg/kg	207.30 ± 2.50	185.65 ± 1.72	190.67 ± 2.51	184.00 ± 3.64
D	Alloxan + Ethanolic extract	100 mg/kg	207.54 ± 2.05	175.51 ± 2.73∗	160.42 ± 2.94∗	152.76 ± 2.94∗
E	Alloxan + Ethanolic extract	200 mg/kg	208.64 ± 2.95	180.39 ± 1.50	183.73 ± 2.73	156.49 ± 1.74∗
F	Alloxan + Ethanolic extract	400 mg/kg	207.30 ± 2.33	186.67 ± 2.65	180.53 ± 4.37	173.77 ± 3.01∗

Values are given as mean ± SEM for groups of six animals each. a Vehicle (0.5% Tween 80 solution in normal saline). b Alloxan single dose of 150 mg/kg i.p in normal saline on day 0. ∗P<0.01 (Dunnet *t*-test), diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

TABLE 3: EFFECT OF ETHANOLIC FRACTION OF THREE PLANTS *ANNONA SQUAMOSA, ECLIPTA ALBA AND BUTEA MONOSPERMA* ON SERUM PROFILE IN ALLOXAN (150 mg/kg i.p.) INDUCED DIABETIC ALBINO RATS AFTER 21 DAYS OF TREATMENT

Treatment	Dose	Serum cholesterol	Serum triglycerides	Serum H.D.L cholesterol	Serum LDL cholesterol	Serum creatinine	Serum urea	Serum alkaline phosphates
A	0.2 mL (a)	140.00 ±5.2	110.23 ±3.8	40.67 ±2.3	87.34 ±5.4	1.38 ±1.3	45.87 ±2.1	120.10 ±9.4
B	0.2 mL(b)	202.32 ±18.3	189.46 ±17.3	42.14 ±1.3	180 ±15.2	2.30 ±1.6	81.13 ±2.9	290 ± 2.7
C	10 mg/kg	186.64 ±5.4∗	118.38 ±6.3	68.54 ±2.5	84.29 ±3.7∗	1.37 ±0.8∗	38.57 ±3.2∗	187 ±3.6∗
D	100 mg/kg	274.29 ±14.5	205 ±5.8	42.39 ±2.8	228 ±10.12	0.64 ±0.4∗	47.76 ±3.4∗	235.64 ±7.6∗
E	200 mg/kg	183.84 ±3.5∗	136.73 ±6.7∗	47.83 ±1.3∗	112.86 ±4.8∗	1.63 ±1.1∗	43.56 ±1.9∗	160.69 ±19.2∗
F	400 mg/kg	143.64 ±7.2∗	120.87 ±5.9∗	48.37 ±2.6∗	99.64 ±5.2∗	1.74 ±1.1∗	37.51 ±1.1∗	143.93 ±7.1∗

Values are given in average body weight (g) ±SEM for groups of six animals each. a Vehicle (0.5% Tween 80 solution in normal saline). b Alloxan single dose of 150 mg/kg i.p in normal saline on day 0. ∗P<0.05 as compared to vehicle control on corresponding day.

Statistical Analysis: All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's *t*-test. Differences between groups were considered significant at ∗P<0.01 levels.

RESULTS:

Anti-Diabetic Study: Effect of compound extract of three plants *annona squamosa, eclipta alba and butea monosperma* extract on fasting blood glucose level in diabetic rats. Ethanolic extract of three plants was subjected to anti-diabetic activity in rats where alloxan monohydrate (120 mg/kg b.w., i.p.) used as the diabetogenic agent. A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats. Ethanolic extract of three plants (at 200 and 400 mg/kg) exhibited a dose dependent significant anti-hyperglycemic activity on 7^{th}, 14^{th} and 21^{st} day post treatment. The extract dose of 100 mg/kg also caused reduction in blood glucose level but the results were found statistically insignificant. The anti-hyperglycemic effect of ethanol extract at was found less effective than the reference standard, Glibenclamide. Glibenclamide produced a significant reduction in blood glucose compare to diabetic control. The results are shown in the **Table 1**.

CONCLUSION: A compound ethanolic fraction of three plants exhibited significant anti-hyperglycemic activities in alloxan induced diabetic rats. This extract has showed improvement in parameters like body weight and lipid profile by enhancing effect on cellular antioxidant defenses to protect against oxidative damage. Present efforts are directed to isolate the active constituents from this fraction and confirmation of mechanism of action.

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CONFLICT OF INTEREST: The authors declare that there was no conflict of interest.

REFERENCES:

1. http://www.who.int/en/
2. http://www.drugs.com/byetta.html
3. Bolen S: Ann Intern Med., 147, 386-99 (2007) [PMID: 17638715]
4. Nahar N: Traditional medicine, Edn.18, Oxford and OBH Publishing Co. Pvt. Ltd., New Delhi 1993: 205-209,
5. Giordano BP, Thrash W, Hollenbaugh L, Dube WP, Hodges C, Swain A, Banion CR and Klingensmith GJ: Performance of seven blood glucose testing systems at high altitude. Diabetes Education 1989; 15: 444-448.
6. Roeschlau P, Bernt E and Gruber W: Enzymatic determination of total cholesterol in serum. Zeitschrift fur Klinische Chemie und Klinische Biochemie 1974; 12: 226.
7. Muller PH, Schmulling RM, Liebich HM and Eggstein M: A fully enzymatic triglyceride determination. Journal of Clinical Chemistry and Clinical Biochemistry 1977; 15: 457-464.
8. Allain CC, Poon LS, Chan CS, Richmond W and Fu PC: Enzymatic determination of total serum cholesterol. Clinical Chemistry 1974; 20: 470-475.

9. Friedewald WT, Levy RI and Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 1972; 18: 499-502.
10. Bowers LD: Kinetic serum creatinine assays I. The role of various factors in determining specificity. Clinical Chemistry 1980; 26: 551-554.
11. Wilson BW: Automatic estimation of urea using urease and alkaline phenol. Clinical Chemistry 1966; 12: 360-368.
12. Sasaki M: A new ultramicro method for the determination of serum alkaline phosphatase. Use of Berthelot's reaction for the estimation of phenol released by enzymatic activity. Igaku to Seibutsugaku 1966; 70: 208-214.

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